E. coli K12 CJ236

1-800-632-7799
info@neb.com
www.neb.com

E4141S 003150220021

E4141S

Lot: 0031502 **Exp: 2/20** **Store at –20°C**

Description: A suspension of *E. coli* strain CJ236 which has been grown in LB medium and brought to 50% glycerol.

Genotype: F∆*(HindIII)::cat* (Tra$^+$ Pil$^+$ Cam^R)/ *ung-1 relA1 dut-1 thi-1 spoT1 mcrA*

Use of Strain CJ236: CJ236 is *dut*$^-$ *ung*$^-$ and contains an F-factor carrying a selectable marker (chloramphenicol resistance). It was made by taking an isolate of BW313 that had spontaneously become F$^-$ and introducing pCJ105, the F´ Cm^R construct (1,2). For use in preparing uracil-containing M13 templates (3,4).

Please Note the Following:

A. Streak out the strain on LB agar containing chloramphenicol (15 µg/ml) to ensure that you start with an F$^+$ host.
B. Do not include chloramphenicol in liquid media: the strain does not grow well.
C. CJ236 is non-suppressing. Do not use M13 vectors that carry amber mutations (mp8, 9 and some mp10 and 11).
D. Store plates at 4°C in the dark.

Preparation of single-stranded uracil-containing template:

1. Transform phagemid vector into CJ236 (expect low transformation efficiency with RbCl or $CaCl_2$ methods).
2. Inoculate 50 ml LB (no amp), containing 0.25 µg/ml uridine, with a fresh colony. Grow at 37°C with vigorous aeration until slightly turbid (< 10 Klett units).
3. Add M13KO7 Helper Phage (NEB #N0315S) to a final concentration of $1x10^8$ pfu/ml. Continue vigorous aeration for 60–90 minutes.
4. Add kanamycin to a final concentration of 70 µg/ml. Grow overnight (14–18 hours) with vigorous aeration.
5. Spin culture at 8,000 rpm for 10 minutes. Transfer supernatant to a new tube and spin again.
6. Pipet upper 90% of supernatant into a new tube. Add 0.2 volume 2.5 M NaCl/20% PEG 8000. Let sit at 4°C for 60 minutes or overnight.
7. Recover phage by centrifugation at 8,000 rpm for 10 minutes. Discard supernatant.
8. Resuspend pellet in 1.6 ml TE (10 mM Tris-HCl, pH 8.0, 1 mM EDTA). Divide into 2 microfuge tubes.
9. Spin in microcentrifuge for 5 minutes to pellet any remaining cells. Transfer supernatants to new tubes.
10. Add 200 µl PEG/NaCl to each. Let sit at room temperature for 5 minutes. Spin in microcentrifuge for 5 minutes.
11. Decant supernatant. Spin briefly. Remove last traces of supernatant with pipetman.
12. Resuspend each pellet in 300 µl TE. Extract with phenol (let sit 15 minutes before spinning), then phenol/chloroform (twice), then chloroform. Add 30 µl 2.5 M NaOAc, pH 4.8 and alcohol precipitate.
13. Suspend dried pellets in 25–50 µl TE. For phagemid with the pUC replication origin, the yield should be > 50 µg single-stranded phagemid. For lower copy number vectors, the bulk of the single-stranded DNA will be helper phage at this point.

Notes: Storage at –70°C is recommended for periods longer than 30 days. Avoid repeated freeze/thaw cycles.

References:

1. Joyce, C. and Grindley, N. (1984) *J. Bact.* 158, 636–643.
2. Raleigh, E. A., Lech, K. and Brent, R. (1989) in Current Protocols in *Molecular Biology eds.* Ausubel, F. M. et al. Publishing Associates and Wiley Interscience; New York. Unit 1.4.
3. Kunkel, T. A., Bebenek, K. and McClary J. (1991) *Methods Enzymol.* 204, 125–139.
4. Kunkel, T. A. et al. (1987) *Methods Enzymol.* 154, 367–382.

CERTIFICATE OF ANALYSIS

E. coli K12 CJ236

1-800-632-7799
info@neb.com
www.neb.com

E4141S 003150220021

E4141S

Lot: 0031502 Exp: 2/20 Store at –20°C

Description: A suspension of *E. coli* strain CJ236 which has been grown in LB medium and brought to 50% glycerol.

Genotype: F∆*(HindIII)::cat* (Tra$^+$ Pil$^+$ Cam^R)/ *ung-1 relA1 dut-1 thi-1 spoT1 mcrA*

Use of Strain CJ236: CJ236 is *dut*$^-$ *ung*$^-$ and contains an F-factor carrying a selectable marker (chloramphenicol resistance). It was made by taking an isolate of BW313 that had spontaneously become F$^-$ and introducing pCJ105, the F´ Cm^R construct (1,2). For use in preparing uracil-containing M13 templates (3,4).

Please Note the Following:

A. Streak out the strain on LB agar containing chloramphenicol (15 µg/ml) to ensure that you start with an F$^+$ host.
B. Do not include chloramphenicol in liquid media: the strain does not grow well.
C. CJ236 is non-suppressing. Do not use M13 vectors that carry amber mutations (mp8, 9 and some mp10 and 11).
D. Store plates at 4°C in the dark.

Preparation of single-stranded uracil-containing template:

1. Transform phagemid vector into CJ236 (expect low transformation efficiency with RbCl or $CaCl_2$ methods).
2. Inoculate 50 ml LB (no amp), containing 0.25 µg/ml uridine, with a fresh colony. Grow at 37°C with vigorous aeration until slightly turbid (< 10 Klett units).
3. Add M13KO7 Helper Phage (NEB #N0315S) to a final concentration of $1x10^8$ pfu/ml. Continue vigorous aeration for 60–90 minutes.
4. Add kanamycin to a final concentration of 70 µg/ml. Grow overnight (14–18 hours) with vigorous aeration.
5. Spin culture at 8,000 rpm for 10 minutes. Transfer supernatant to a new tube and spin again.
6. Pipet upper 90% of supernatant into a new tube. Add 0.2 volume 2.5 M NaCl/20% PEG 8000. Let sit at 4°C for 60 minutes or overnight.
7. Recover phage by centrifugation at 8,000 rpm for 10 minutes. Discard supernatant.
8. Resuspend pellet in 1.6 ml TE (10 mM Tris-HCl, pH 8.0, 1 mM EDTA). Divide into 2 microfuge tubes.
9. Spin in microcentrifuge for 5 minutes to pellet any remaining cells. Transfer supernatants to new tubes.
10. Add 200 µl PEG/NaCl to each. Let sit at room temperature for 5 minutes. Spin in microcentrifuge for 5 minutes.
11. Decant supernatant. Spin briefly. Remove last traces of supernatant with pipetman.
12. Resuspend each pellet in 300 µl TE. Extract with phenol (let sit 15 minutes before spinning), then phenol/chloroform (twice), then chloroform. Add 30 µl 2.5 M NaOAc, pH 4.8 and alcohol precipitate.
13. Suspend dried pellets in 25–50 µl TE. For phagemid with the pUC replication origin, the yield should be > 50 µg single-stranded phagemid. For lower copy number vectors, the bulk of the single-stranded DNA will be helper phage at this point.

Notes: Storage at –70°C is recommended for periods longer than 30 days. Avoid repeated freeze/thaw cycles.

References:

1. Joyce, C. and Grindley, N. (1984) *J. Bact.* 158, 636–643.
2. Raleigh, E. A., Lech, K. and Brent, R. (1989) in Current Protocols in *Molecular Biology eds.* Ausubel, F. M. et al. Publishing Associates and Wiley Interscience; New York. Unit 1.4.
3. Kunkel, T. A., Bebenek, K. and McClary J. (1991) *Methods Enzymol.* 204, 125–139.
4. Kunkel, T. A. et al. (1987) *Methods Enzymol.* 154, 367–382.

CERTIFICATE OF ANALYSIS